

[0221]

TABLE 2

Sample	ATP signal in RLUs	ATP signal Capture efficiency (%)
<i>E. coli</i> ( $1 \times 10^4$ cells) control (100% signal)	30,866	N/A
<i>E. coli</i> ( $1 \times 10^5$ cells) control (100% signal)	176,933	N/A
CM-111 pellet from $\sim 1 \times 10^3$ /ml sample	27,589	89
CM-111 pellet from $\sim 1 \times 10^4$ /ml sample	94,840	54

N = 2, Std deviation <10%, Data normalized to water alone (16,464 RLUs) background for *E. coli* controls and normalized to unreacted CM-111 (41,424 RLUs) background for the CM-111 pellets contacted with bacteria.

[0222] From above example it can be seen that the particulate capture agents can be used to concentrate bacteria from an aqueous sample.

## Example 8

Concentration of *E. coli* Using CM-111 Using a Type II Device

[0223] An isolated *E. coli* (ATCC 33090) colony was inoculated from a streak plate into 5 ml Tryptic Soy Broth (, TSB, Becton Dickinson, Sparks, Md.) and incubated at 37° C. for 18-20 hours. This overnight culture at approximately  $10^8$  colony forming units/ml was diluted in sterile-filtered deionized water (MilliQ, Millipore, MA) and spiked in 10 ml of sterile-filtered deionized water to obtain final concentration of  $10^3$ /ml (approximately  $10^4$  cfus total). The spiked water was added to the device already containing 10 mg pre-sterilized (121 deg C., 15 minutes) powder of CM-111 (Cosmetic Microspheres-111, 3M Company, St Paul) and 100 microliters of the 100× Adsorption Buffer. The device was sealed with surface sterilized Parafilm and placed on a rocking platform. The capped devices were then incubated at room temperature (25° C.) for 1 and 9 minutes (total elapsed=time 2 mins and 10 minutes) on a Thermolyne Vari Mix™ rocking platform (Barnstead International, Iowa, 14 cycles/minute).

[0224] After the incubation the Parafilm was removed and the plunger with the was inserted into the housing until it contacted the frangible seal. By inserting the plunger further, to break the frangible seal, the *E. coli* bound CM-111 was transferred to the lower receptacle of the housing along with approximately 100 microliters of the liquid sample. Control tubes containing *E. coli* without microparticles were treated similarly.

[0225] The CM-111 pellet was retrieved by cutting open the lower receptacle the particles were transferred to a 1.5 ml sterile microfuge tube. A 100 microliter volume of the BacTiter-Glo™ reagent (Promega, Madison, Wis.) was added to the pellet, mixed by vortexing for 5 seconds on a VWR Fixed Speed Vortex Mixer (3200 rpm for 5 seconds) and read on a tabletop luminometer (FB12 Single Tube Luminometer, Berthold Detection Systems USA, Oak Ridge, Tenn.). For 100% signal, a 100 microliter volume from a  $10^5$ /ml dilution was used. Results were calculated using the formula below and tabulated in Table 3 below:

$$\text{ATP Signal \% Capture efficiency} = \frac{(\text{RLUs on CM-111 pellet} / \text{RLUs from about } 10^4 \text{ total } E. coli) \times 100}{100}$$

RLU=Relative Luciferase Units.

[0226]

TABLE 3

Sample	ATP signal in RLUs	ATP signal Capture efficiency (%)
<i>E. coli</i> ( $10^4$ cells) control	96,544	N/A
Water sample with <i>E. coli</i> (no concentration)	25,583	0
CM-111 pellet with concentrated <i>E. coli</i> 2 min testing time	56,932	59
CM-111 pellet with concentrated <i>E. coli</i> 10 min testing time	58,543	61

N = 2, Std deviation <10%, Data normalized to water alone (27,938 RLUs) background for *E. coli* controls and normalized to unreacted CM-111 (30,611 RLUs) background for the CM-111 pellets contacted with bacteria.

## Example 9

Concentration of *E. coli* Using AB-CM-111 Using a Type II Device

[0227] A 10 mg aliquot of AB-CM (Adsorption buffer treated CM-111) was also tested for concentration of *E. coli* from 10 ml water using the procedure described in Example 8. The contact time was 9 minutes, 1 min to settle AB-CM using the POREX plunger. The data is tabulated in Table 4.

TABLE 4

Sample	ATP signal in RLUs	ATP signal Capture efficiency (%)
<i>E. coli</i> ( $10^4$ ) control	82,845	N/A
Water sample with <i>E. coli</i> (no concentration)	733	1
AB-CM pellet with concentrated <i>E. coli</i>	44,105	53

N = 2, Std deviation <10%, Data normalized to water alone (20,281 RLUs) background for *E. coli* controls and normalized to unreacted AB-CM (44,488 RLUs) background for the CM-111 pellets contacted with bacteria.

[0228] From above example it can be seen that the particulate capture agents can be used to concentrate bacteria from an aqueous sample.

## Example 10

## Comparative Example

Detection of *E. coli* in Unconcentrated Samples

[0229] State-of-the-art water testing comprises a method where 100 microliters of water is tested for ATP using a standard ATP bioluminescence assay (for example, 3M CLEANTRACE Water—Free ATP Cat. No. AQF100, available from 3M Company, St. Paul, Minn.).

[0230] An overnight culture of *E. coli* (ATCC 33090) in tryptic soy broth was diluted in sterile water to produce two suspensions. Suspension A contained approximately  $10^3$  CFU/ml and Suspension B contained about  $10^5$  CFU/ml. One hundred microliter aliquots of each suspension were mixed with 100 microliter volumes of the BacTiter-Glo™ reagent (Promega, Madison, Wis.) and the resulting bioluminescence was measured with a luminometer as described in Example 8. The results are presented in Table 5.